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CSIR NET – Life Science

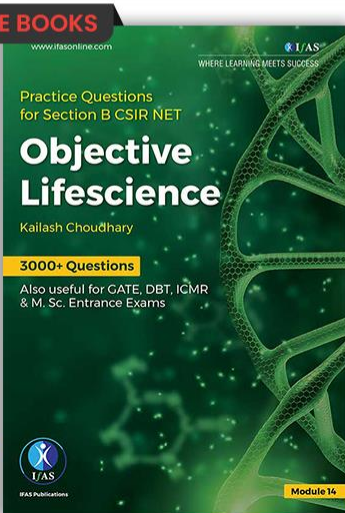
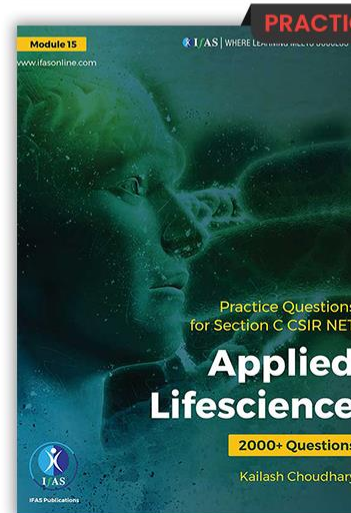
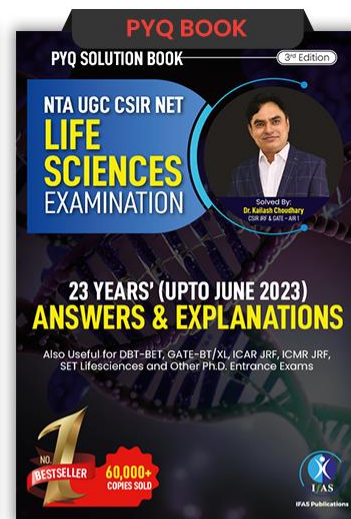
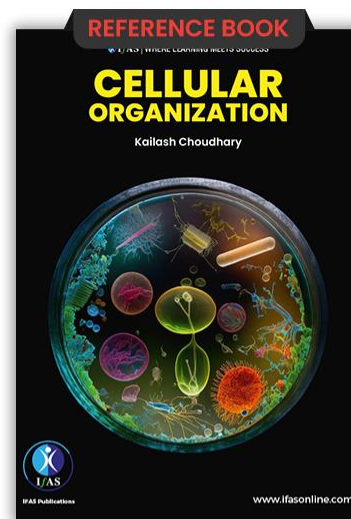
Unit 2: Cellular Organization

L5

**Active Membrane
Transport**



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Points to be covered in this Lecture



Active Membrane Transport



Primary active transport



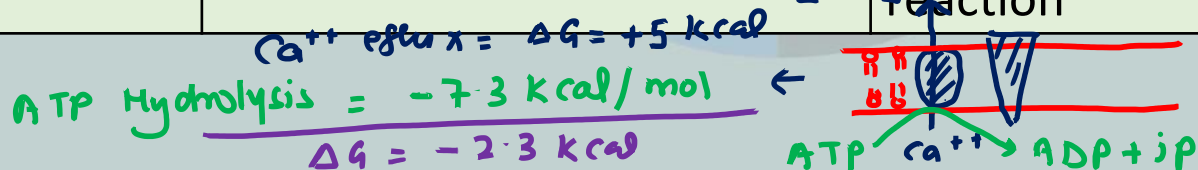
P-type, V-type, F-type, ABC pumps,
group translocation



Secondary active transport -
symport, antiport

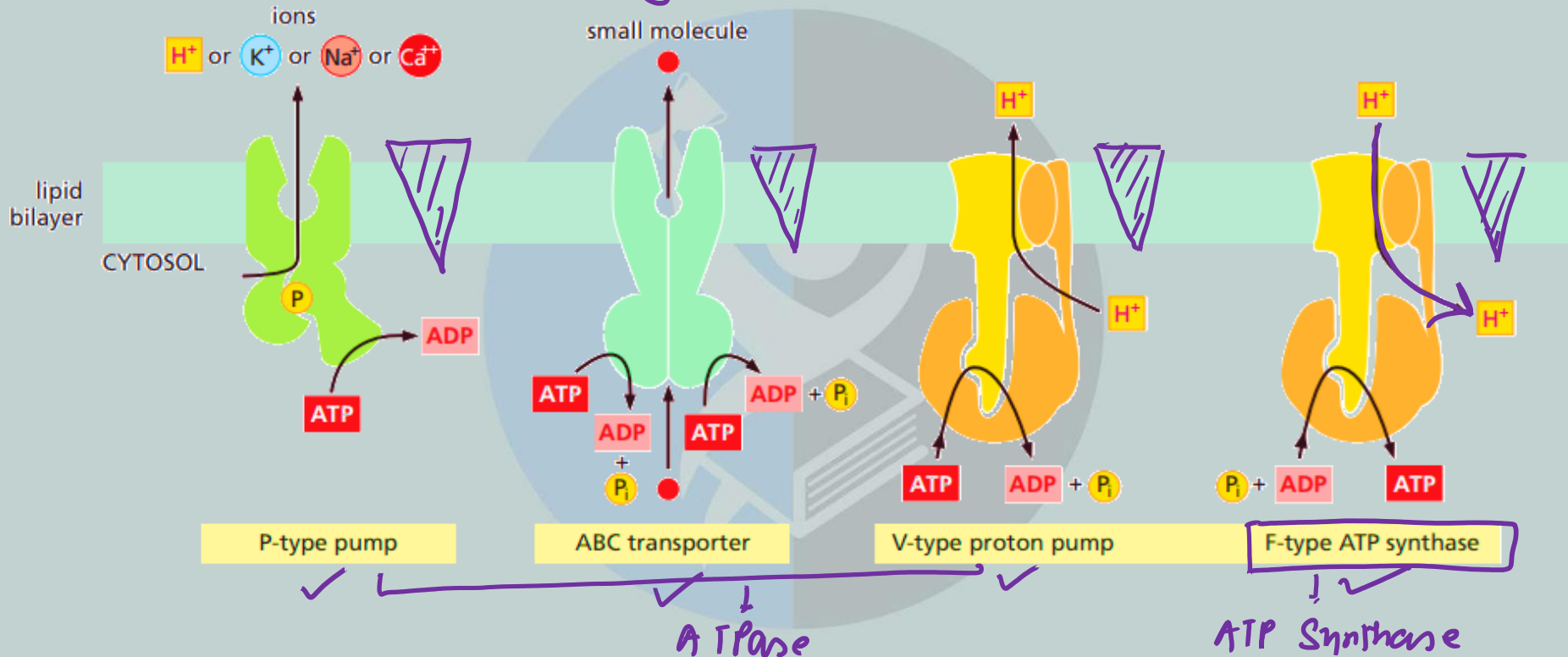
Active and Passive Transport

	Passive transport	Active Transport
Transport	Along gradient	Against gradient
Energy	Not needed ✓	Needed, ATP or <u>gradient</u> , other energy <u>source</u>
Transporter ✓	Not needed for diffusion. Needed for facilitated diffusion	<u>Needed</u> , termed as <u>pumps</u> Uphill process (nonspontaneous reaction) Coupled with a spontaneous reaction





≠ Pump - use ATP as Energy Source





P-type

- Get phosphorylated at conserved Aspartic acid residue
- Example : Na^+/K^+ pump, Ca^{2+} pump, Flippase, H^+ , K^+ Pump, H^+ Pump

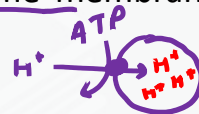
ATP dependent Pumps

F-type

- Present on mitochondrial inner membrane, bacterial plasma membrane and thylakoid membrane of chloroplast.
- Pump proton against gradient or use gradient to produce ATP → act as ATPase or ATP Synthase

V-type

- Present on vacuolar membrane, lysosome, endosome membrane, golgi and plasma membrane of osteoclasts
- V-ATPases acidify these intracellular organelles



The ATP-binding cassette (ABC) transporter

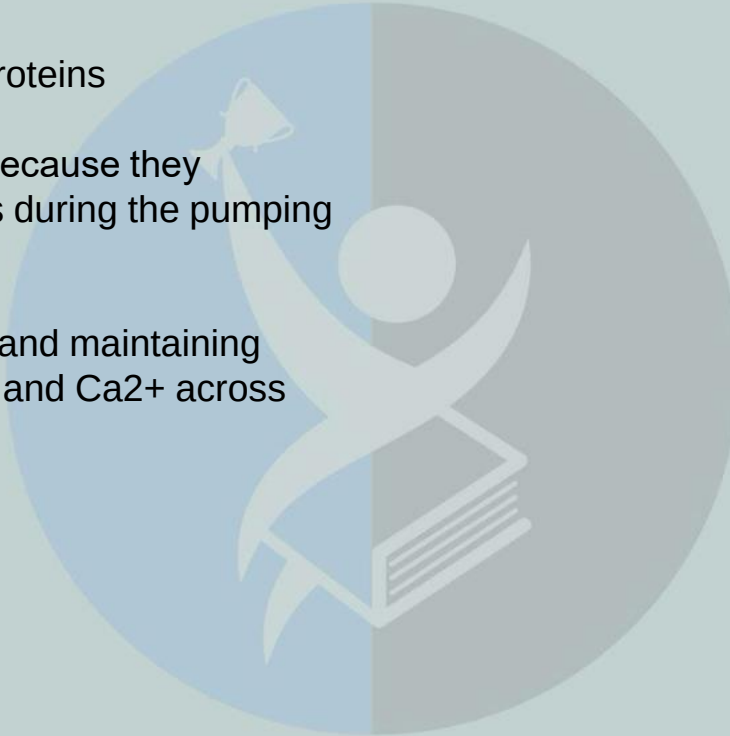
- ✓ Larest and oldest transport system superfamily
- They are present in archaea, eubacteria and eukaryotes.
- Examples includes multidrug resistance (MDR) transport protein of cancer cells which provides resistance againsts anti-cancer agents such as Colchicine, Vinblastine, Etoposide, Daunomycin, Adriamycin and Vincristine.

• Flippase - ABC Transporter

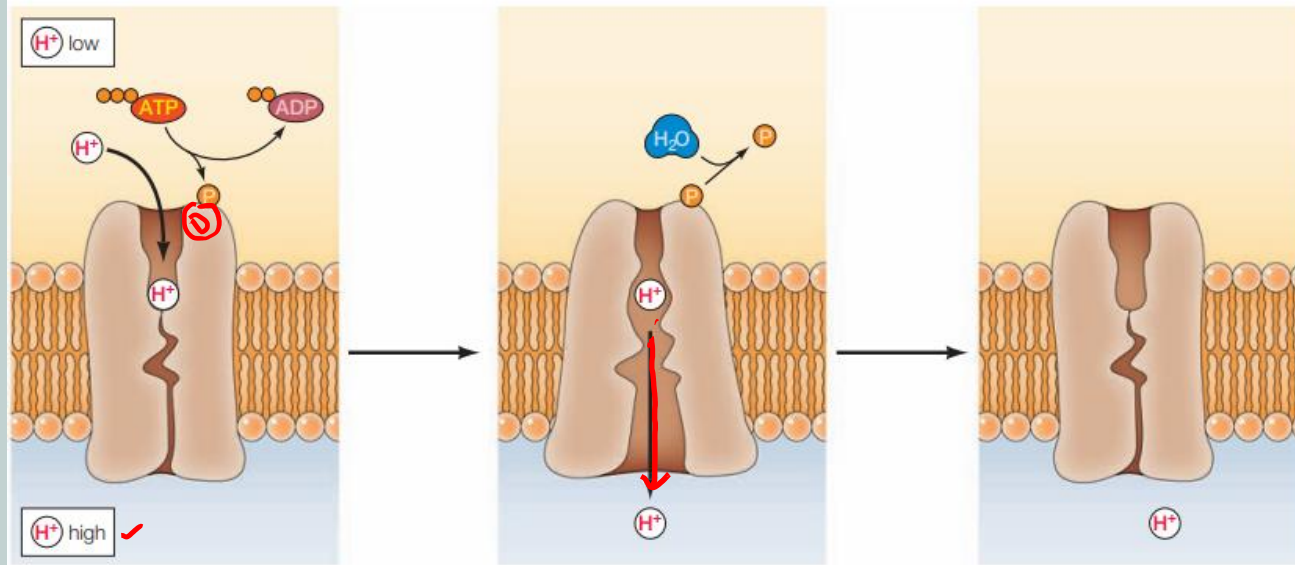


P-type pumps

- Multi pass transmembrane proteins
- They are called “P-type” because they phosphorylate themselves during the pumping cycle
- responsible for setting up and maintaining gradients of Na^+ , K^+ , H^+ , and Ca^{2+} across cell membranes



Model of active transport

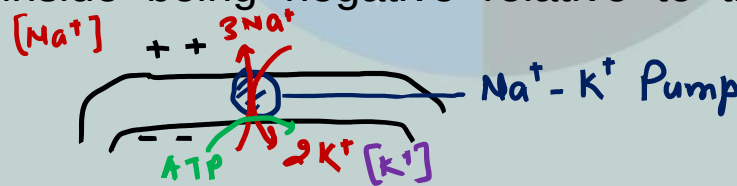


- Binding of H^+ is accompanied by phosphorylation of the carrier protein, which induces a conformational change that drives H^+ transport against the electrochemical gradient.
- Release of H^+ and hydrolysis of the bound phosphate group then restores the carrier to its original conformation



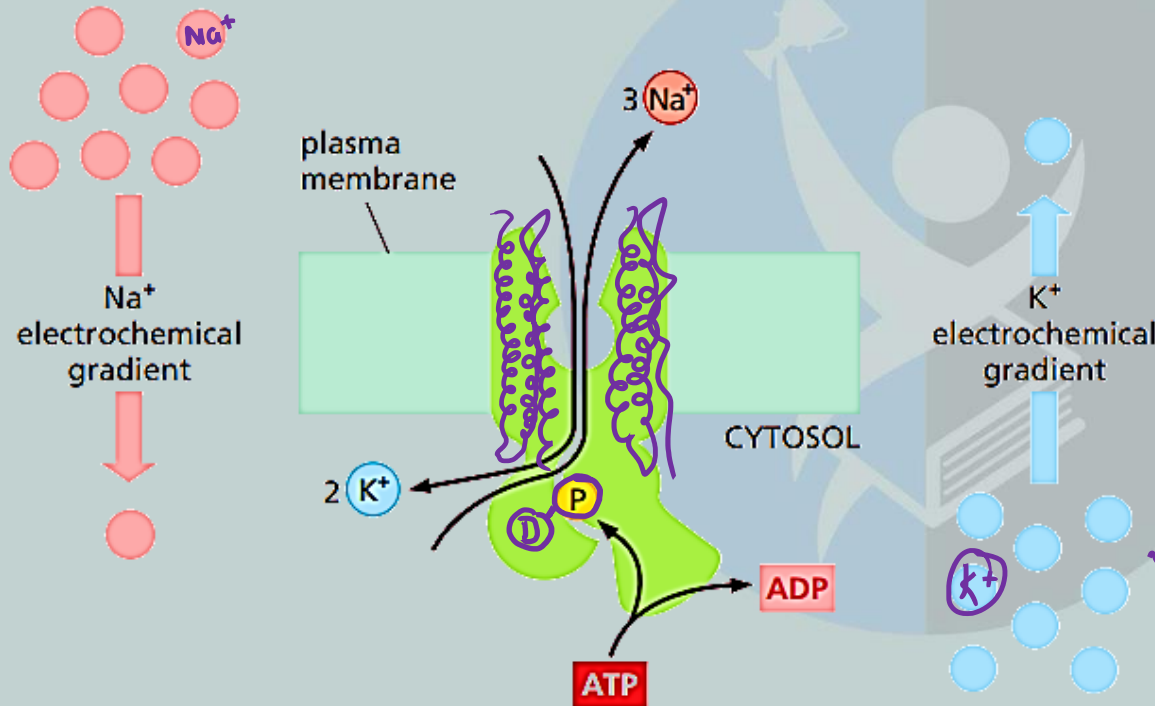
① $\text{Na}^+ - \text{K}^+$ pump

- Na^+ gradient produced by the $\text{Na}^+ - \text{K}^+$ pump drives the transport of most nutrients into animal cells and also has a crucial role in regulating cytosolic pH & cell volume.
- A typical animal cell devotes almost one-third of its energy to fuelling this pump ($\approx 33\% \text{ Energy}$)
- it is electrogenic: it drives a net electric current across the membrane, tending to create an electrical potential
- ✓ with the cell's inside being negative relative to the outside.





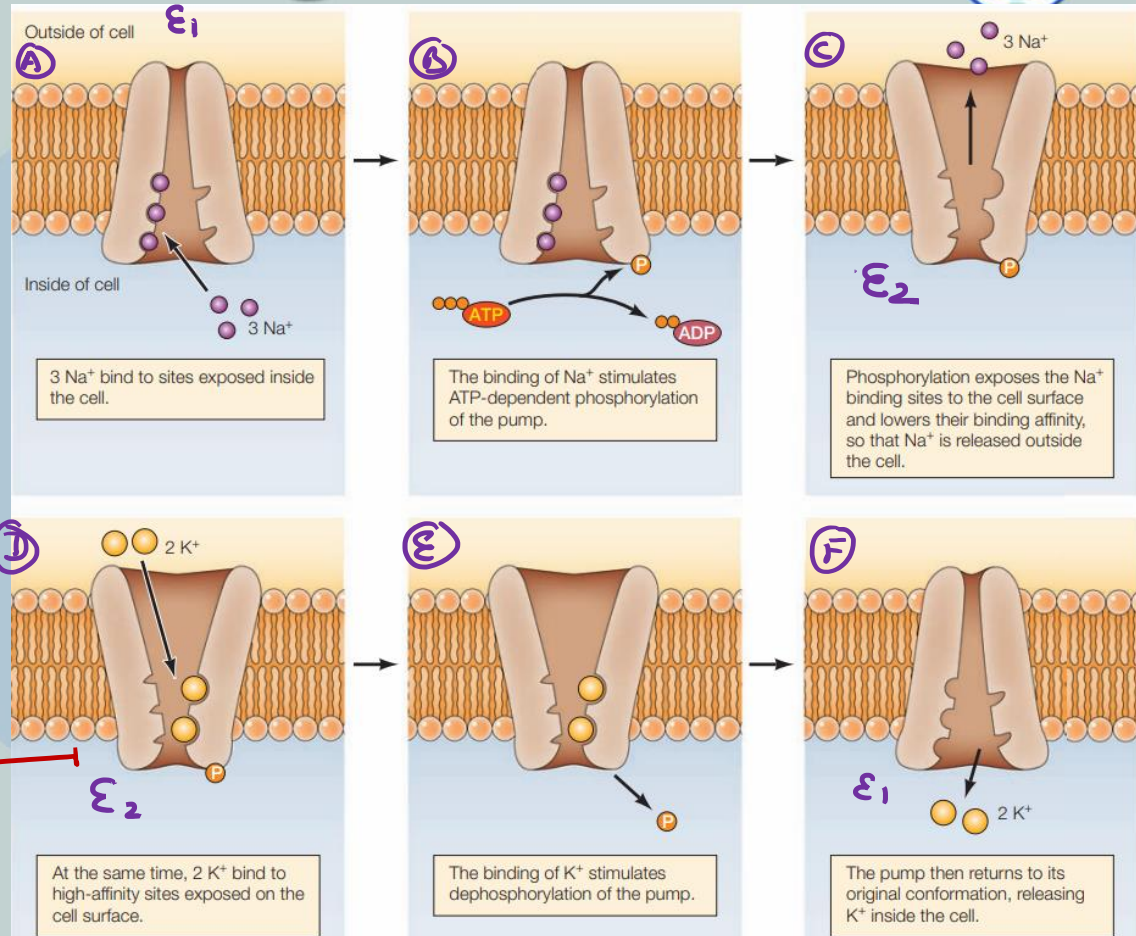
The function of the $\text{Na}^+\text{-K}^+$ pump.



- This p-type ATPase actively pumps Na^+ out of and K^+ into a cell against their electrochemical gradients.
- For every molecule of ATP hydrolyzed by the pump, three Na^+ are pumped out and two K^+ are pumped in.
- An aspartate is phosphorylated and dephosphorylated during the pumping cycle

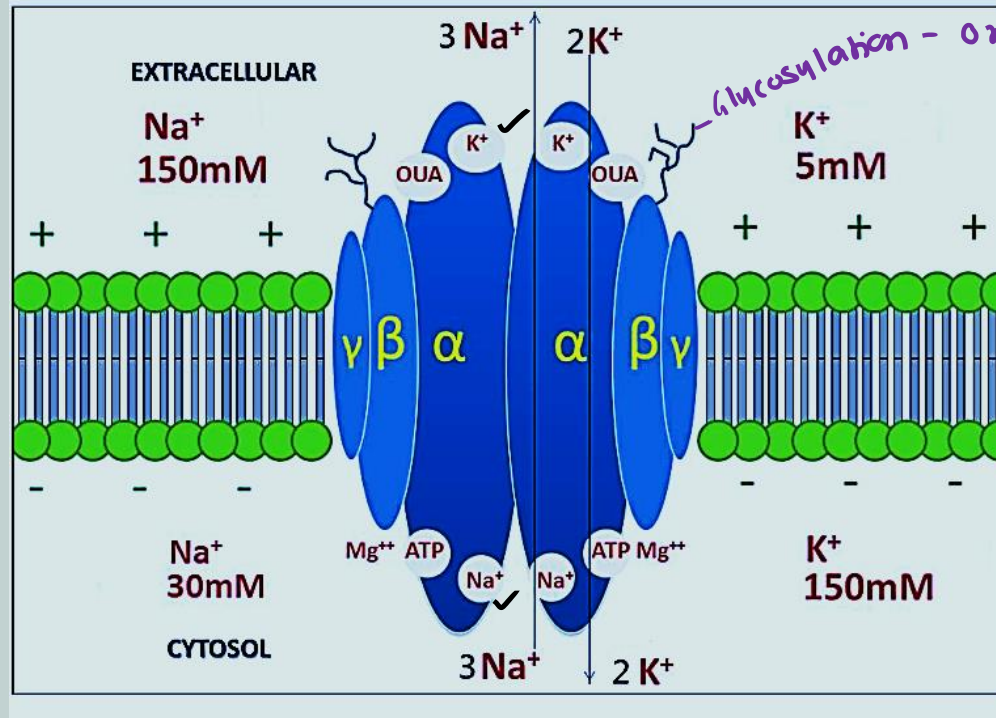


Model for operation of the Na⁺-K⁺ pump





Structure



glycosylation - on β subunit

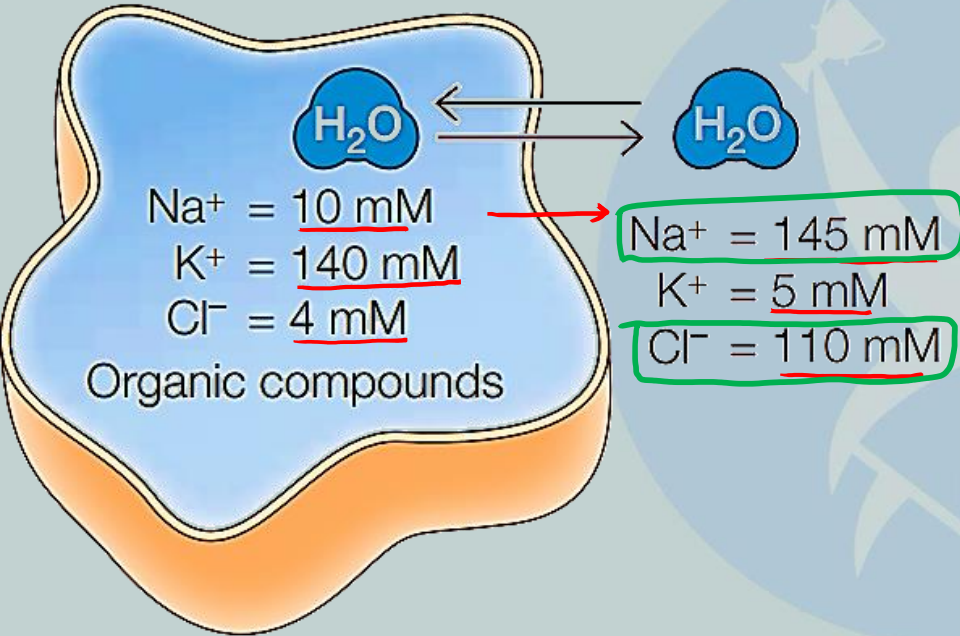
α-Subunit

- Na⁺ Binding
- K⁺ Binding
- ATP Binding
- Ovabain (Toxin)

Na⁺-K⁺ Pump
in E₂P state



Na⁺-K⁺ pump is inhibited by Cardiac Glycosides such as Ovabain and Digitalis



Ion gradients across the plasma membrane of a typical mammalian cell-

- The low concentrations of Na^+ and Cl^- balance the high intracellular concentration of organic compounds, equalizing the osmotic pressure and preventing the net influx of water



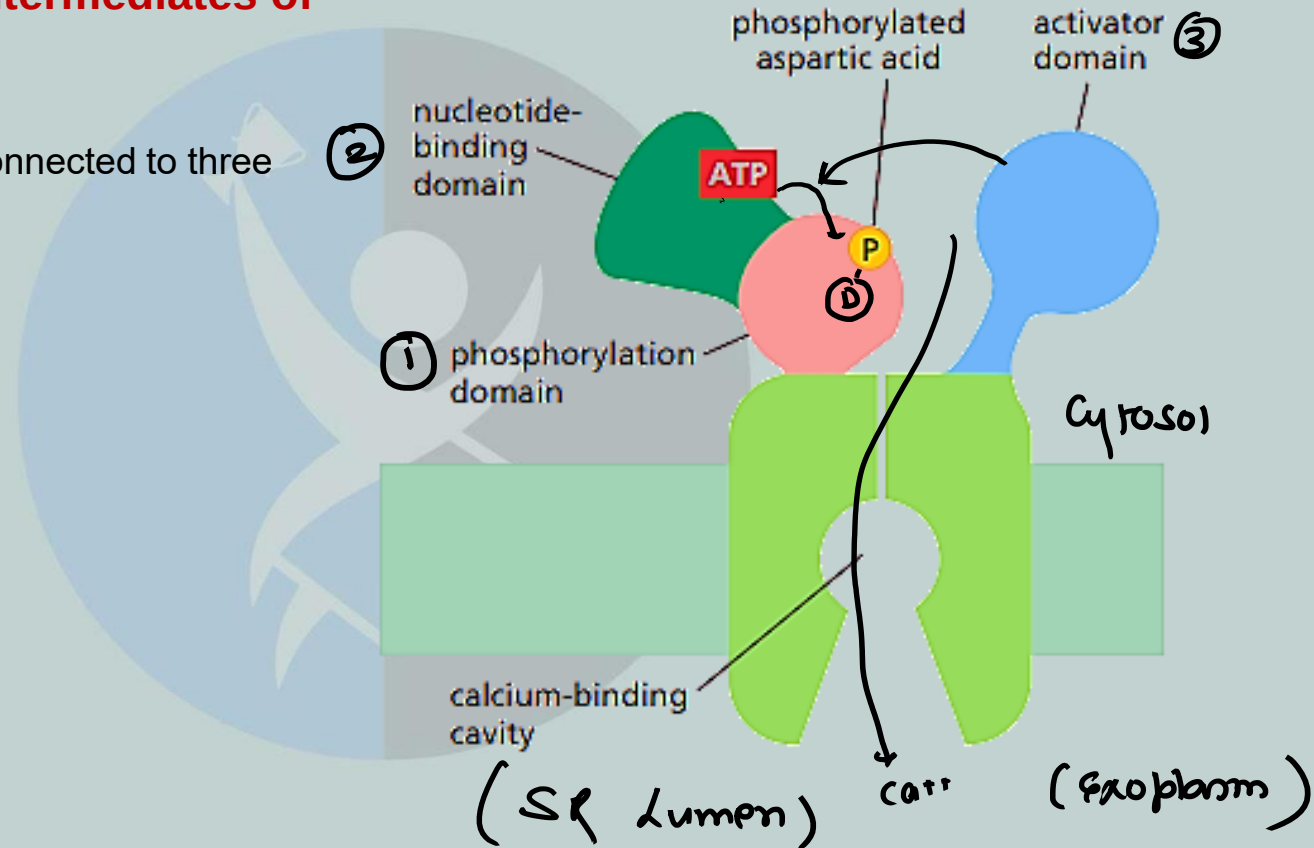
② Ca^{2+} pump, or Ca^{2+} ATPase -

- in the sarcoplasmic reticulum (SR) membrane of skeletal muscle cells is a well-understood P-type transport ATPase.
- SR serves as an intracellular store of Ca^{2+} .
↳ (SER)
- When an action potential depolarizes the muscle cell plasma membrane, Ca^{2+} is released into the cytosol from the SR through Ca^{2+} -release channels.
- stimulating the muscle to contract.
- The Ca^{2+} pump, on SER moves Ca^{2+} from the cytosol back into the SR.
- The endoplasmic reticulum of non-muscle cells contains a similar Ca^{2+} pump, but in smaller quantities. ✓

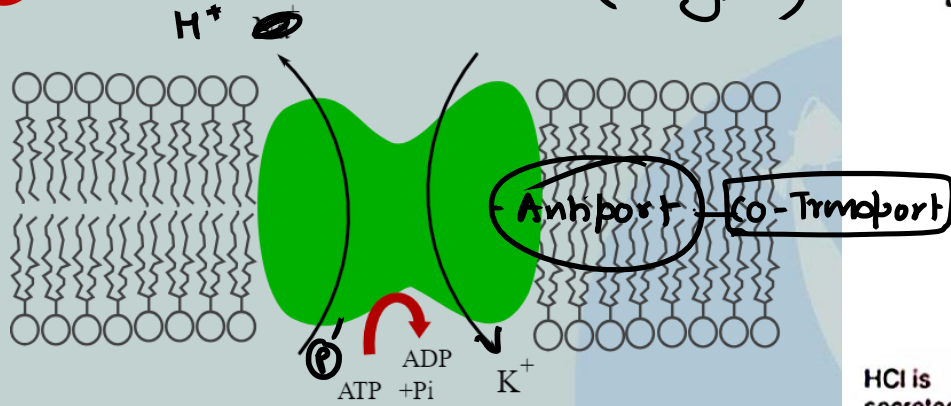


Structures of transport intermediates of the SR Ca^{2+} pump-

10 transmembrane α helices connected to three cytosolic domains ✓

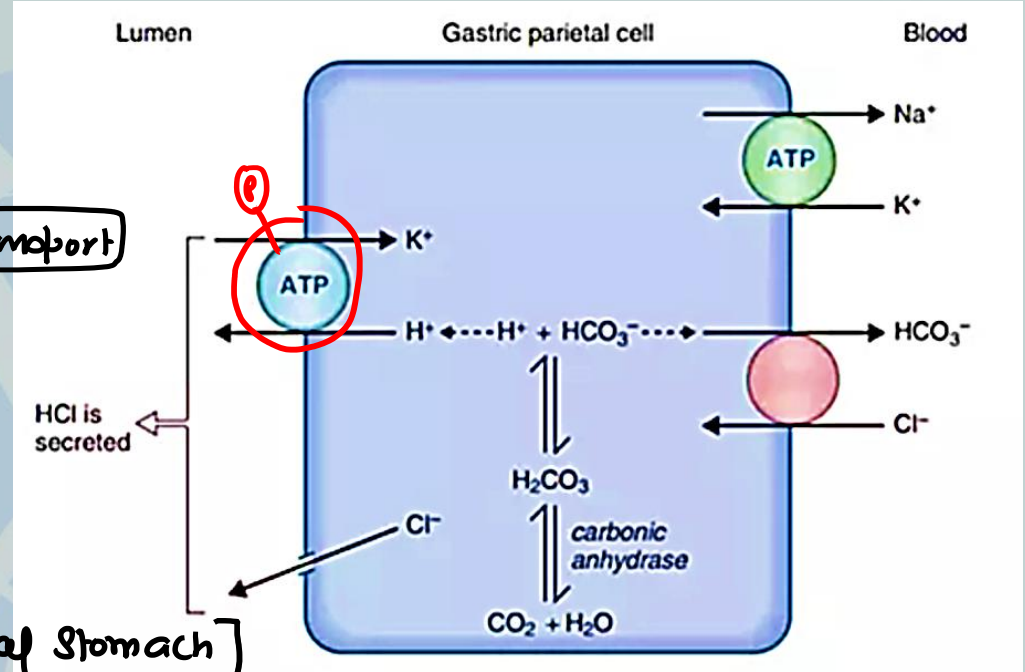


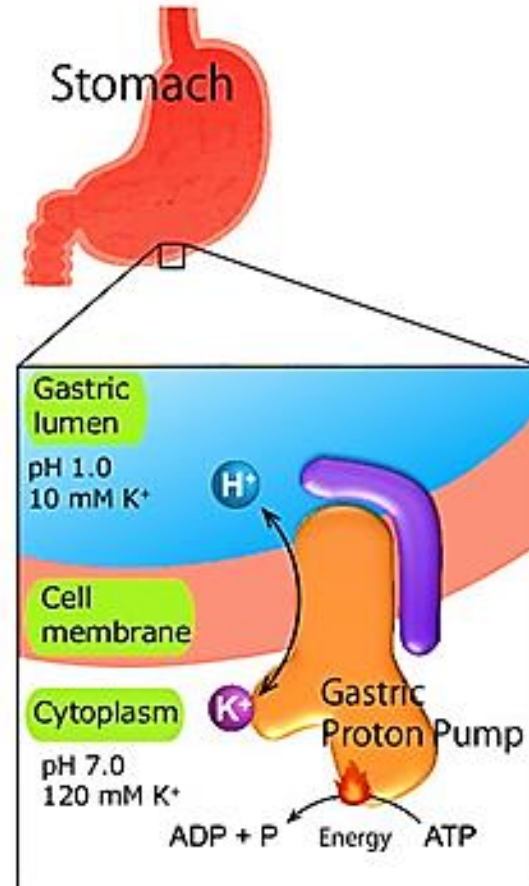
③ Potassium proton pump (P-Type)



- The key player in acid secretion is a H^+/K^+ ATPase or "proton pump" located on membrane. [Parietal cell of Stomach] $\rightarrow$ Acid Secretion.

- This ATPase is magnesium-dependent, and not inhibitable by ouabain.

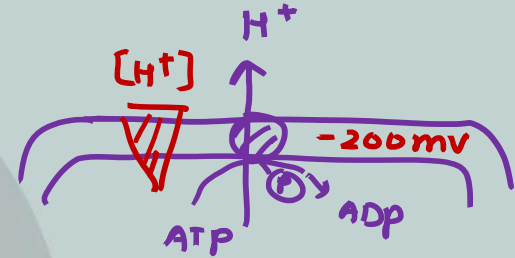






④ Proton pump – P type of pump

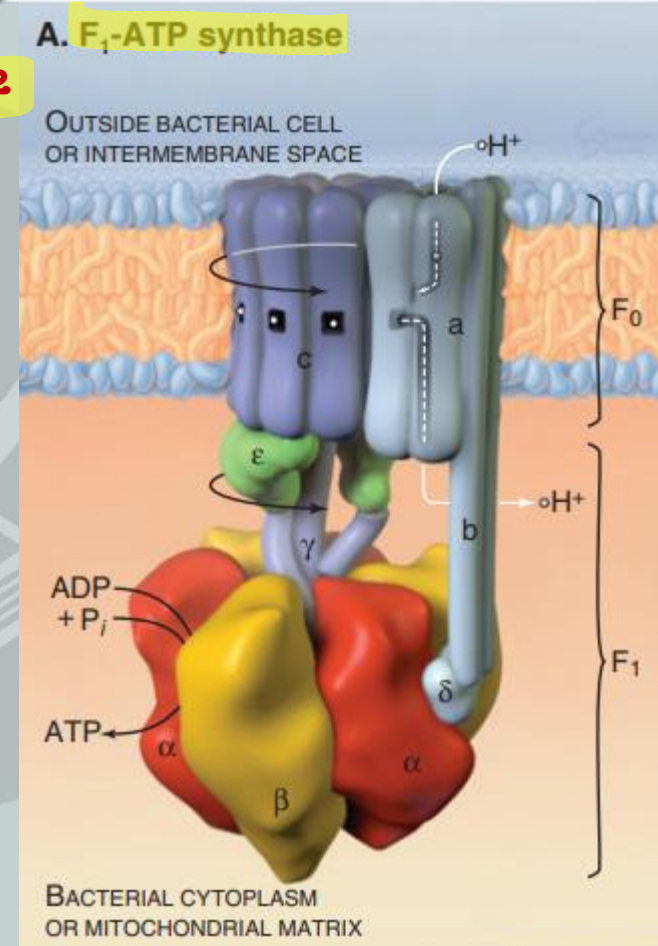
- Present on bacterial and plant plasma membrane
- Major electrogenic pump in plant and bacteria
- Generate -200 mv membrane potential in plant and bacterial plasma membrane



2. F type Pump/F Type ATPase/ATPSynthase

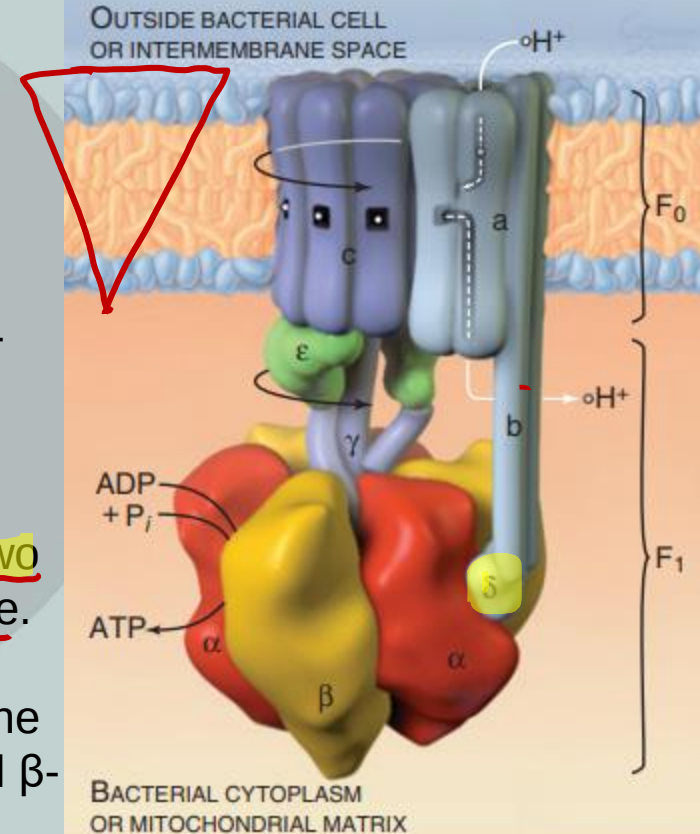
- ✓ • H⁺ pump
- Discovered by Fernandez
- Present on
 - inner mitochondrial membrane
 - Bacterial plasma membrane
 - Thylakoid membrane
- Rotational catalysis occurs in F Type pump

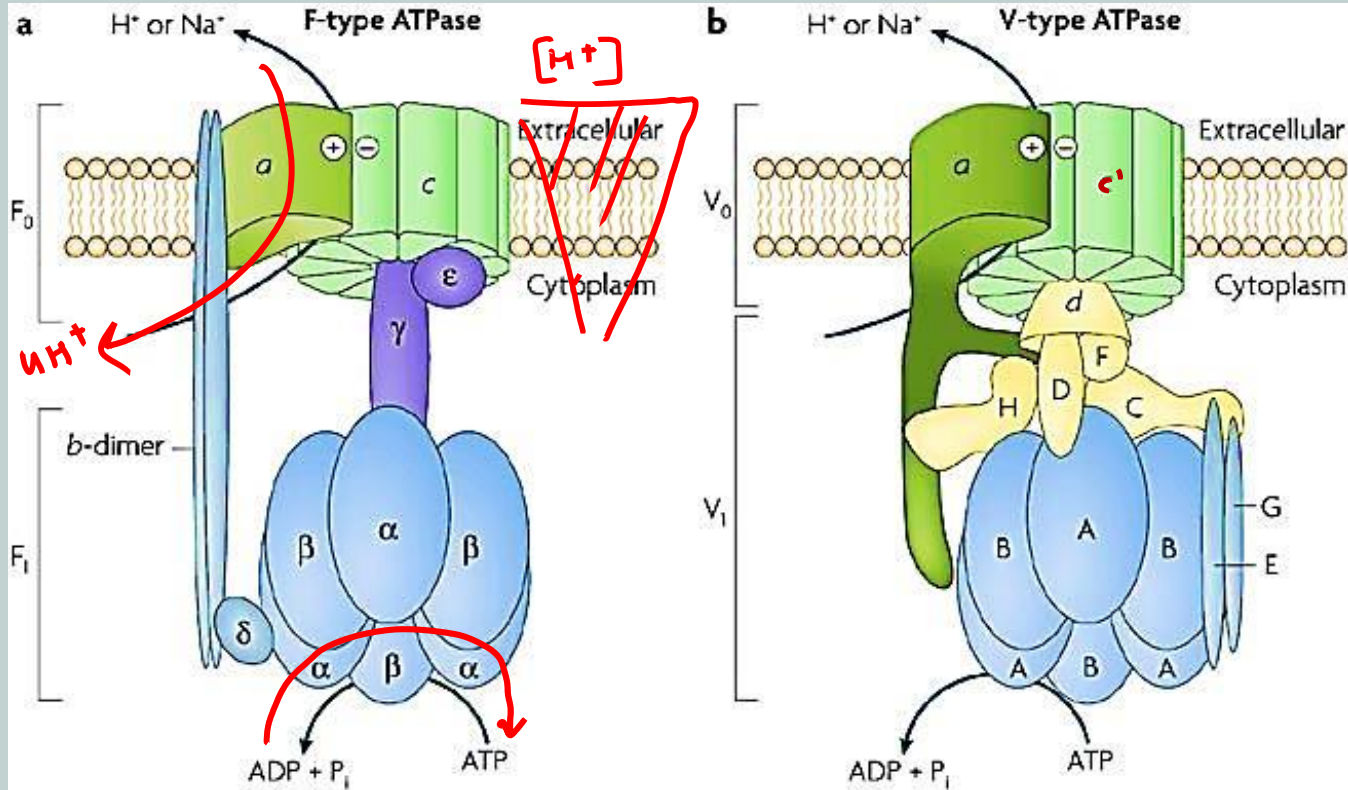
↓
ATP Synthesis

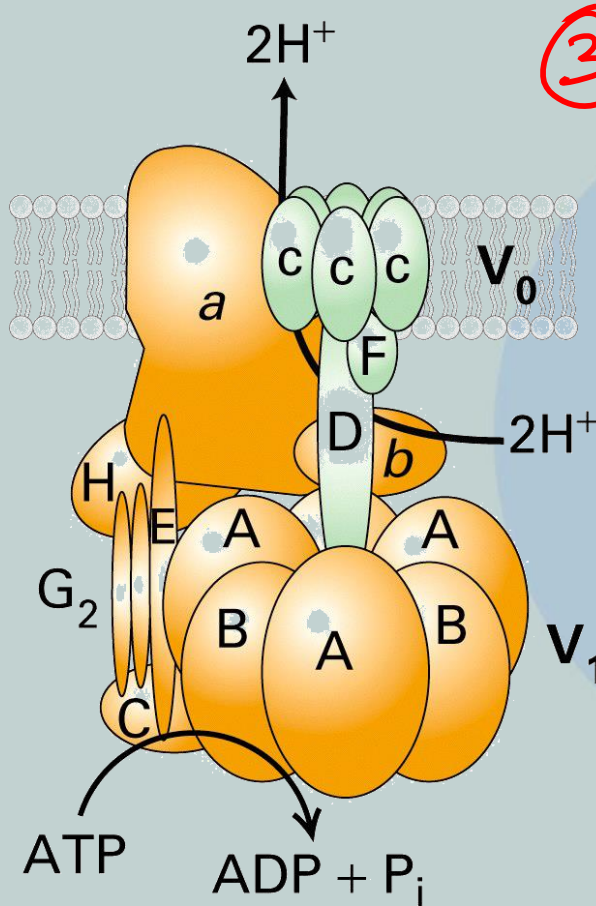


- The mitochondrial F_0 complex has a single “b” subunit with two transmembrane strands.
- The **c-subunits** are hairpins of two α -helices
- Eight to 15 c-subunits (depending on the species)
- The **single a-subunit** is closely apposed to the ring of c-subunits and **linked to the α - and β -subunits** by one of three peripheral shafts.
- The **a-subunit** consists of multiple α -helices that form two half channels for protons to move across the membrane.
- ~~Other protein subunits attach the ring of c-subunits to the γ -subunit camshaft that rotates inside the ring of α - and β -subunits.~~

A. F_1 -ATP synthase







③ V-class proton pumps

Vacuolar membranes in plants, yeast, other fungi

Endosomal and lysosomal membranes in animal cells

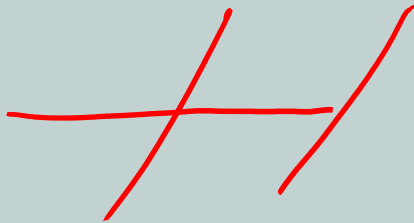
Plasma membrane of osteoclasts and some kidney tubule cells

Bone phagocytotic cells

-> Always act as ATPase



- These pumps transport H^+ only. ✓
- found in lysosomes, endosomes and ✓
plant vacuoles.
- transport H^+ ions to make the lumen or ✓
inside of the lysosome acidic (pH 4.5 -
5.0)



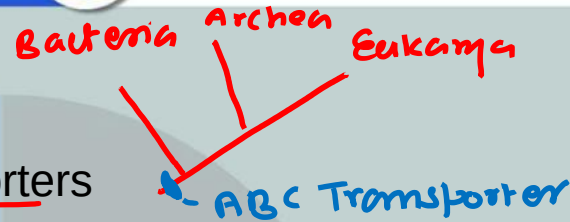


- V-type ATPases are composed of subunits homologous to other rotary ATPases .
- The A- and B-subunits are homologs of F-type α - and β -subunits.
- Three peripheral stalks anchor the channel forming an a-subunit to the hexamer of A- and B-subunits.
- An ancient gene duplication made the c-subunits of eukaryotic V-type pump twice as large as those of F-type ATPases and archaeal A-type ATPases.
- Each of 10 c-subunits has four α -helices with one conserved aspartic acid residue to transfer the proton.
- V-type ATPase typically hydrolyzes three ATPs to transport 10 protons.



④ ABC transporters *and oldest*

- The largest family of membrane transporters
- highly conserved ATP-binding domains or ATP-binding cassettes (ABC)
- More than 100 family members have been identified in both prokaryotic and eukaryotic cells.
- Different members of the family function either as importers that bring nutrients into cells (in prokaryotes)
- export toxic substances out of cells (in both prokaryotes and eukaryotes).





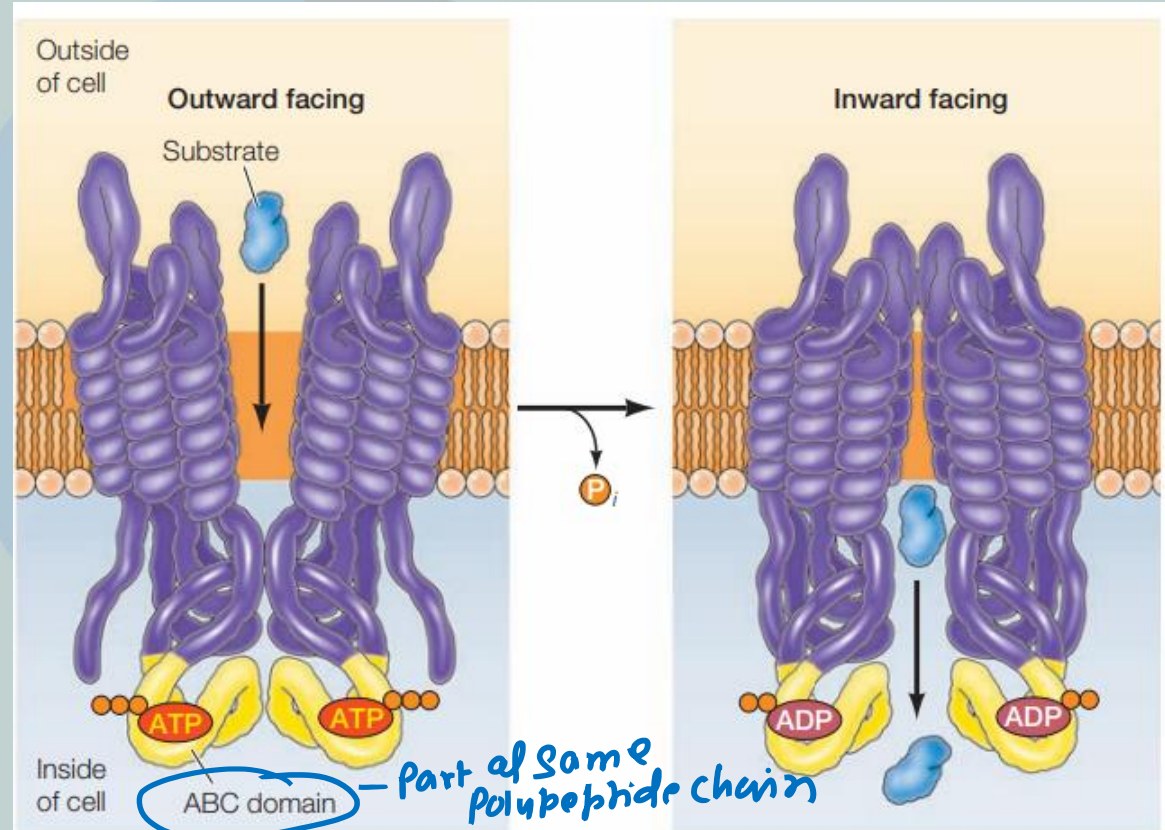
The ABC transporters are composed of two ATP-binding domains and two transmembrane domains, each containing six transmembrane α helices

Model of active transport by an ABC transporter

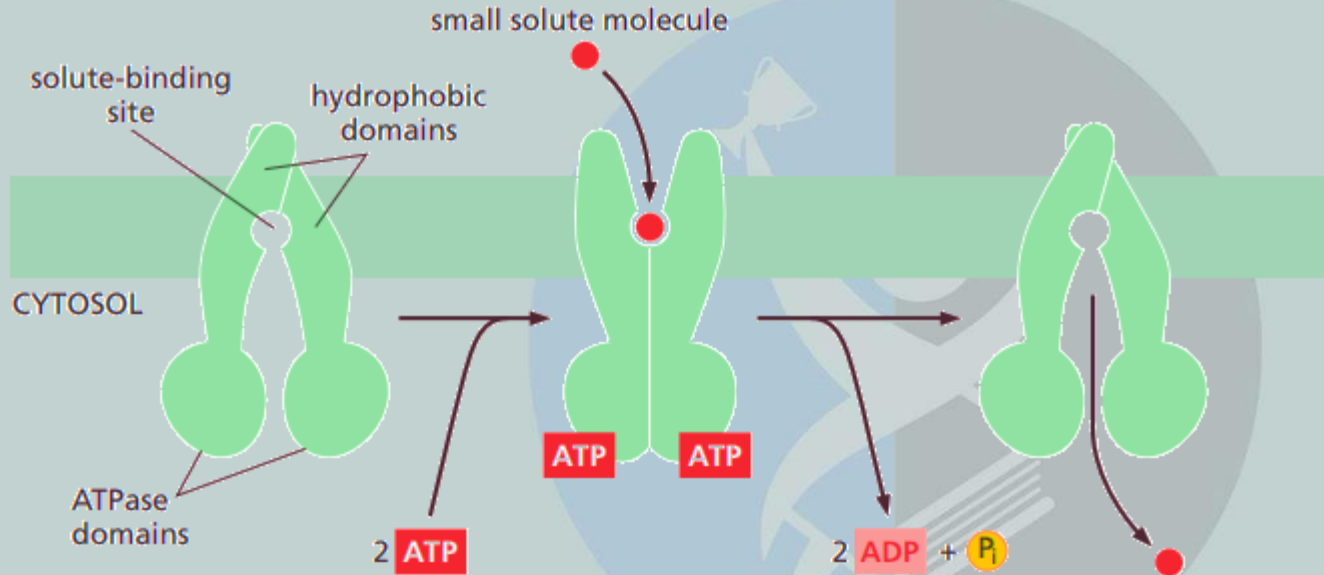
two cytosolic ATP-binding domains (ABC domains).

ATP bound to the ABC domain.

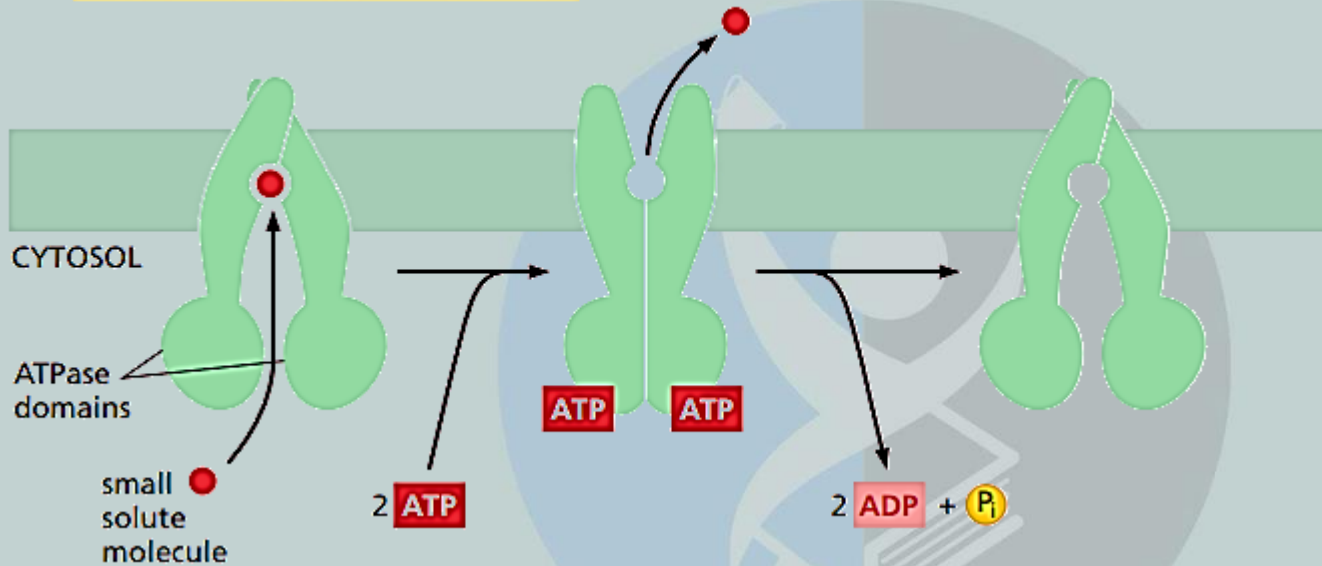
Hydrolysis of ATP to ADP changes the conformation of the transporter, releasing its substrate inside the cell.



(A) A BACTERIAL ABC TRANSPORTER



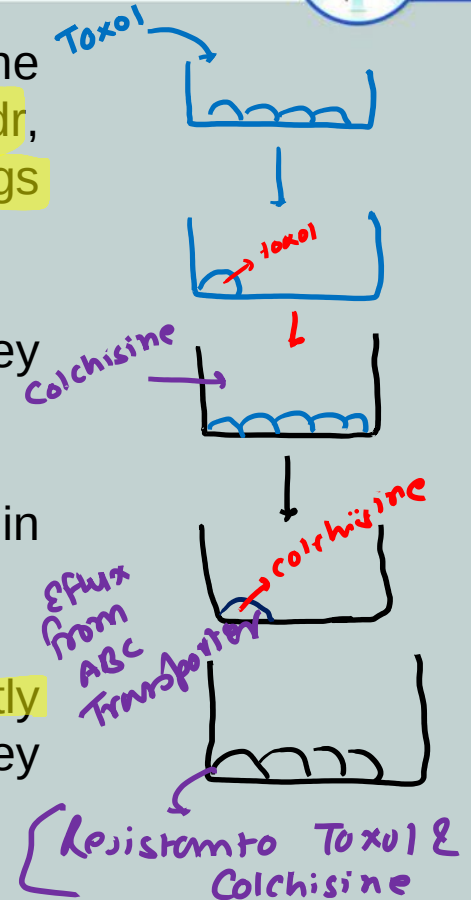
(B) A EUKARYOTIC ABC TRANSPORTER



In ABC transporter – ABC Domain always present in cytosolic side

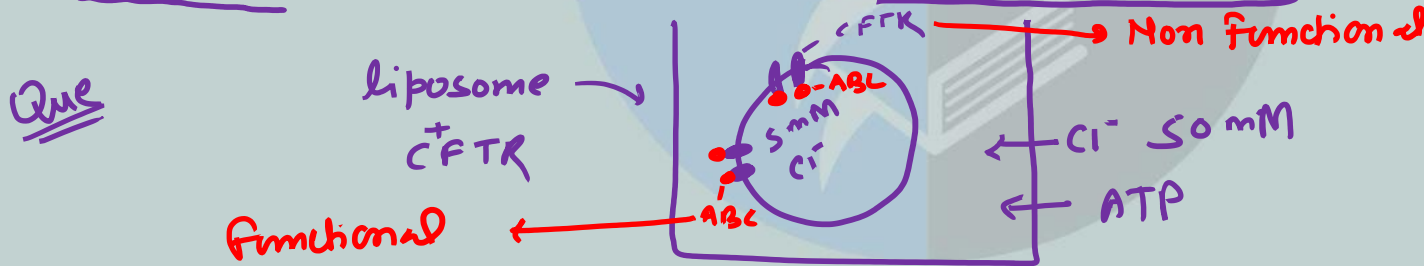


- The first eukaryotic ABC transporter was discovered as the product of a gene (called the multidrug resistance, or *mdr*, gene) that makes cancer cells resistant to a variety of drugs used in chemotherapy
- They are normally expressed in a variety of cells, where they function to remove potentially toxic foreign compounds.
- cells of the brain appears to play an important role in protecting the brain from toxic chemicals.
- Unfortunately, the MDR transporters are frequently expressed at high levels in cancer cells, where they recognize a variety of drugs and pump them out of cells



Cystic fibrosis transmembrane conductance regulator, or CFTR

- functions as a Cl⁻ channel in epithelial cells, and defective Cl⁻ transport is characteristic of the disease.
- Most cases of cystic fibrosis are the result of a mutation in CFTR that interferes with proper folding of the protein



If CFTR topology is changed (inside vesicle) then ATP can not binds with ABC domain and CFTR fails to transfer



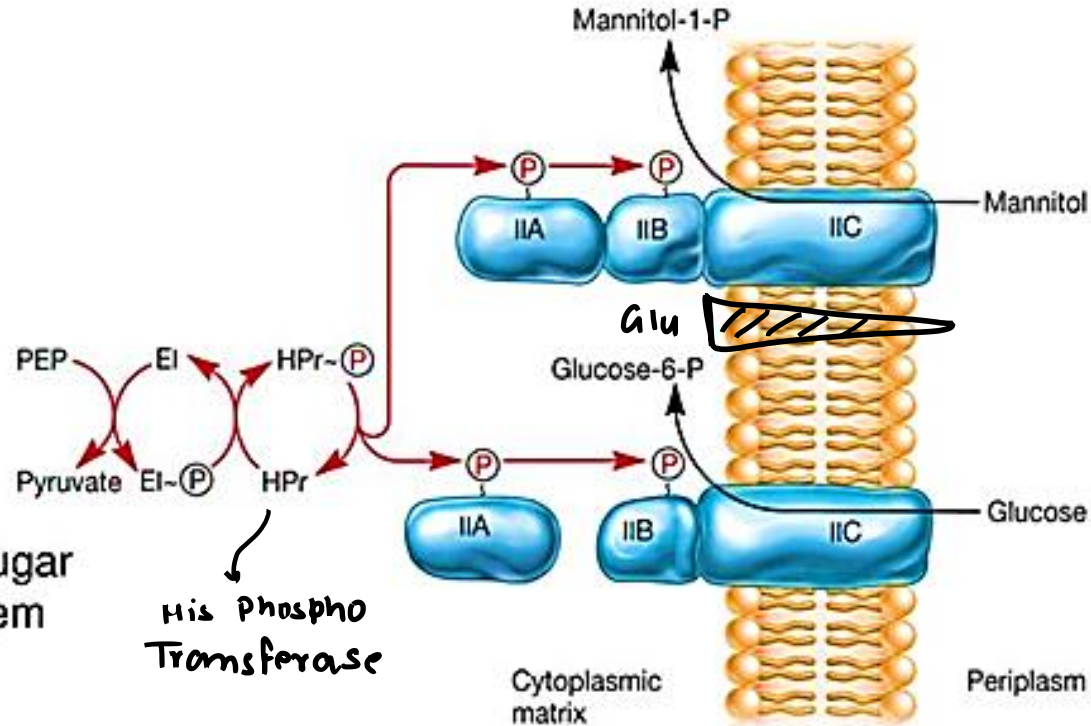
Group Translocation energy-dependent process
Phosphoenol pyruvate:

PEP is out as
Energy Source

- **sugar Phosphotransferase system** PTS Sugars
- Group **Translocation** Low to high Energy is used
Transport protein
chemically modifies molecule as it is brought into cell
↓
(Glucose)



Group Translocation $\rightarrow$ +nt in Prokaryotes



Phosphoenol pyruvate: sugar
Phosphotransferase system
PTS
Sugars

His Phospho
Transferase

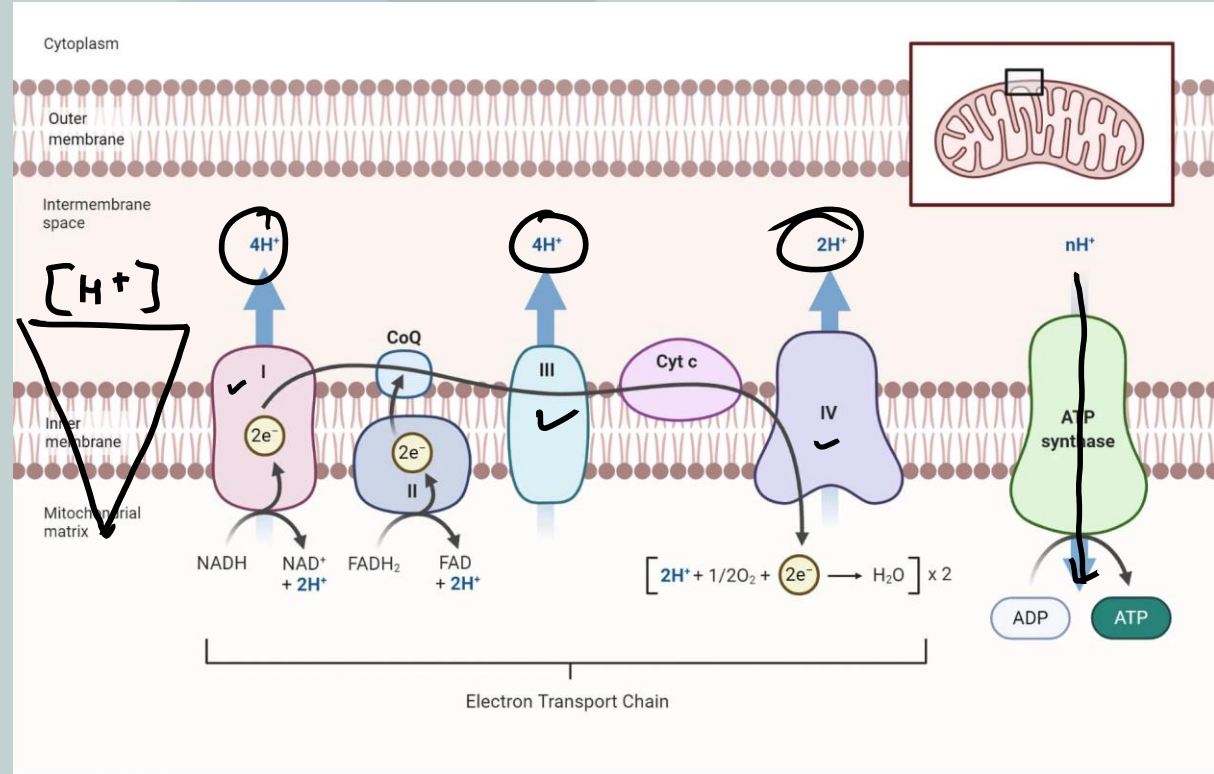
Electron flow used as energy source

Electron transport chain

mt inner membrane

Generate electron motif force

H^+ Pump out



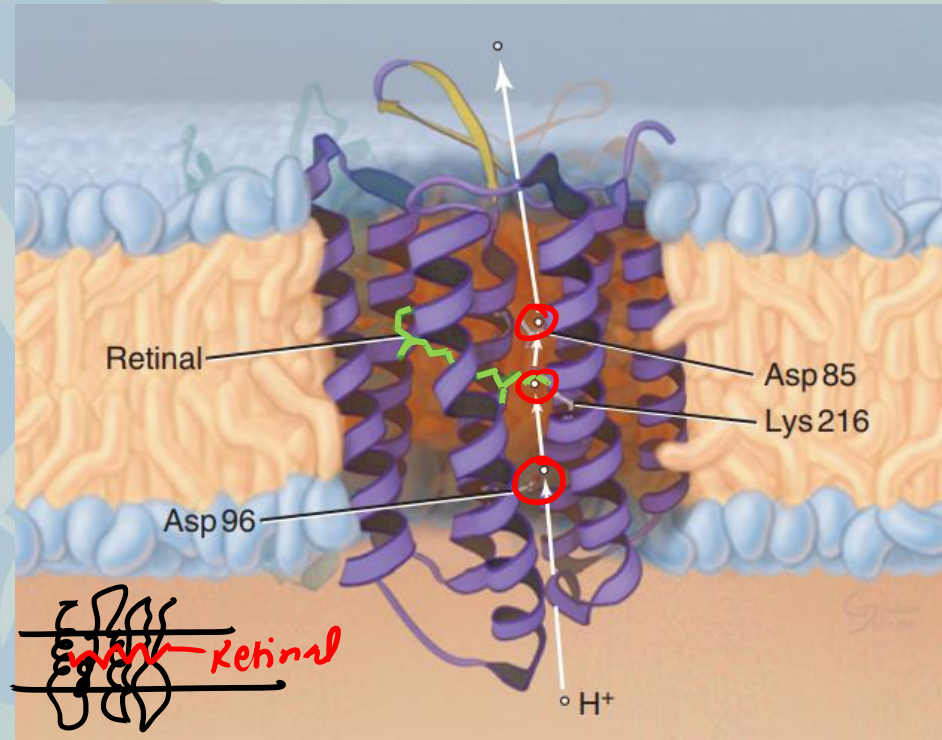
Light-Driven Proton Pumping by Bacteriorhodopsin

Bacteriorhodopsin

- Act as proton pump
- Present in halobacterium
- Span 7 times to the membrane

Absorption of light by retinal drives conformational changes in the protein that favor the transfer of the proton across the membrane up its concentration gradient

(Light act as Energy Source)





The mechanism starts with retinal in the all-trans configuration

[Dark]

↓ *in light*

Absorption of a photon isomerizes retinal to the 13-cis configuration

↓
Bacteriorhodopsin activate → act as H^+ pump.

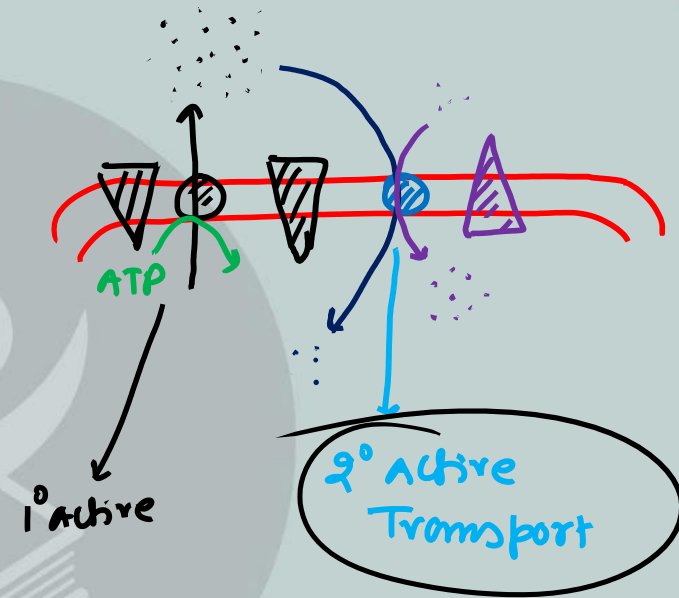


Secondary Active Transport

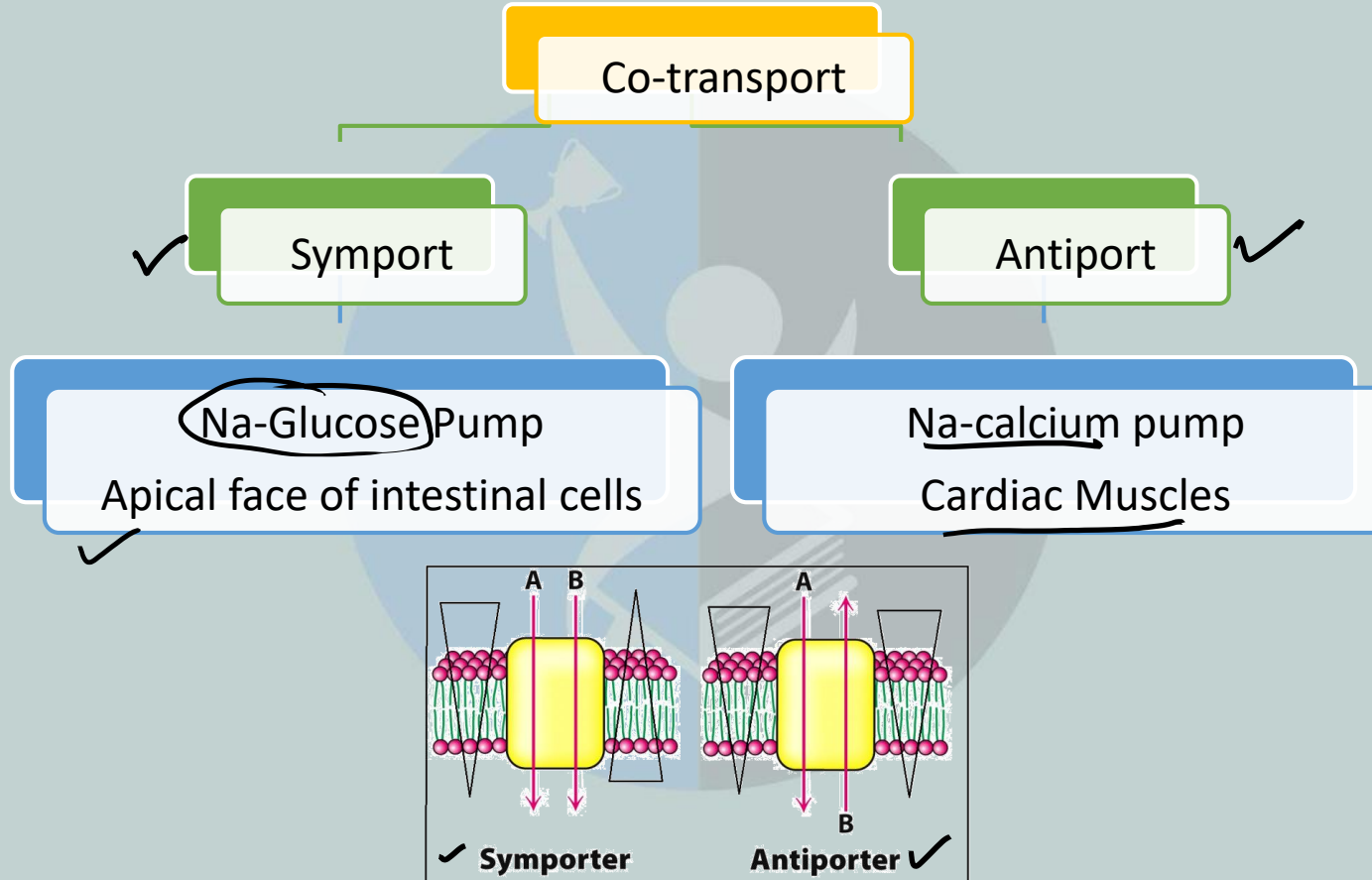
Utilizes electrochemical gradient generated by primary active transport as energy input.

Dissipates electrochemical gradient

Example: $\text{Na}^+ - \text{Ca}^{2+}$ Pump



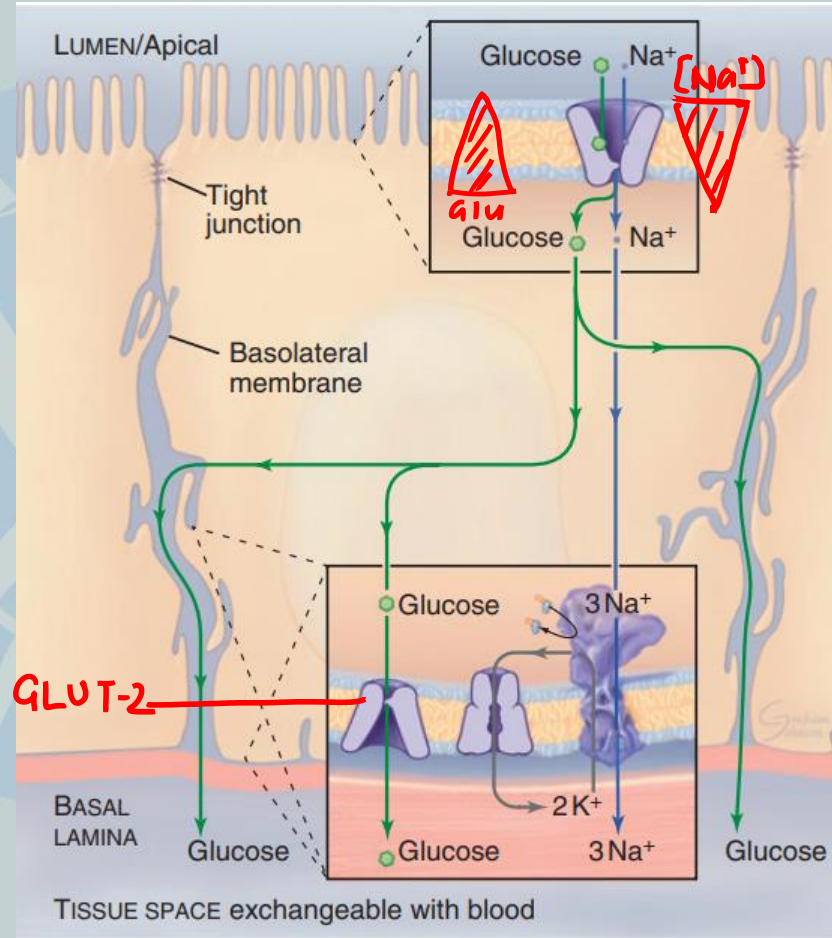
Energy Source - Gradient Energy



Na⁺ glucose transporter

Present on apical surface of intestinal epithelial cell

- Uptake glucose by using Na⁺ gradient
- SGLT1 (sodium glucose cotransporter 1) Na⁺ / glucose symporter, restricted to the apical plasma membrane

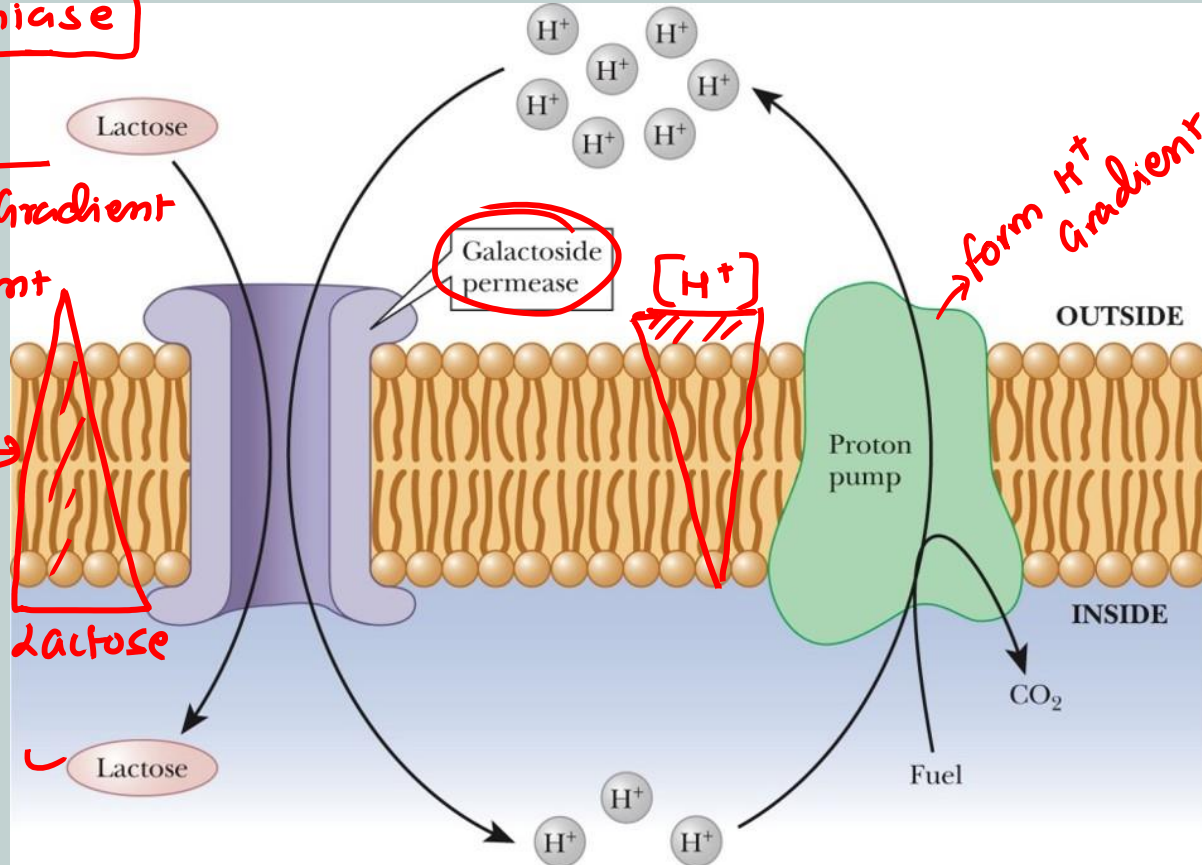


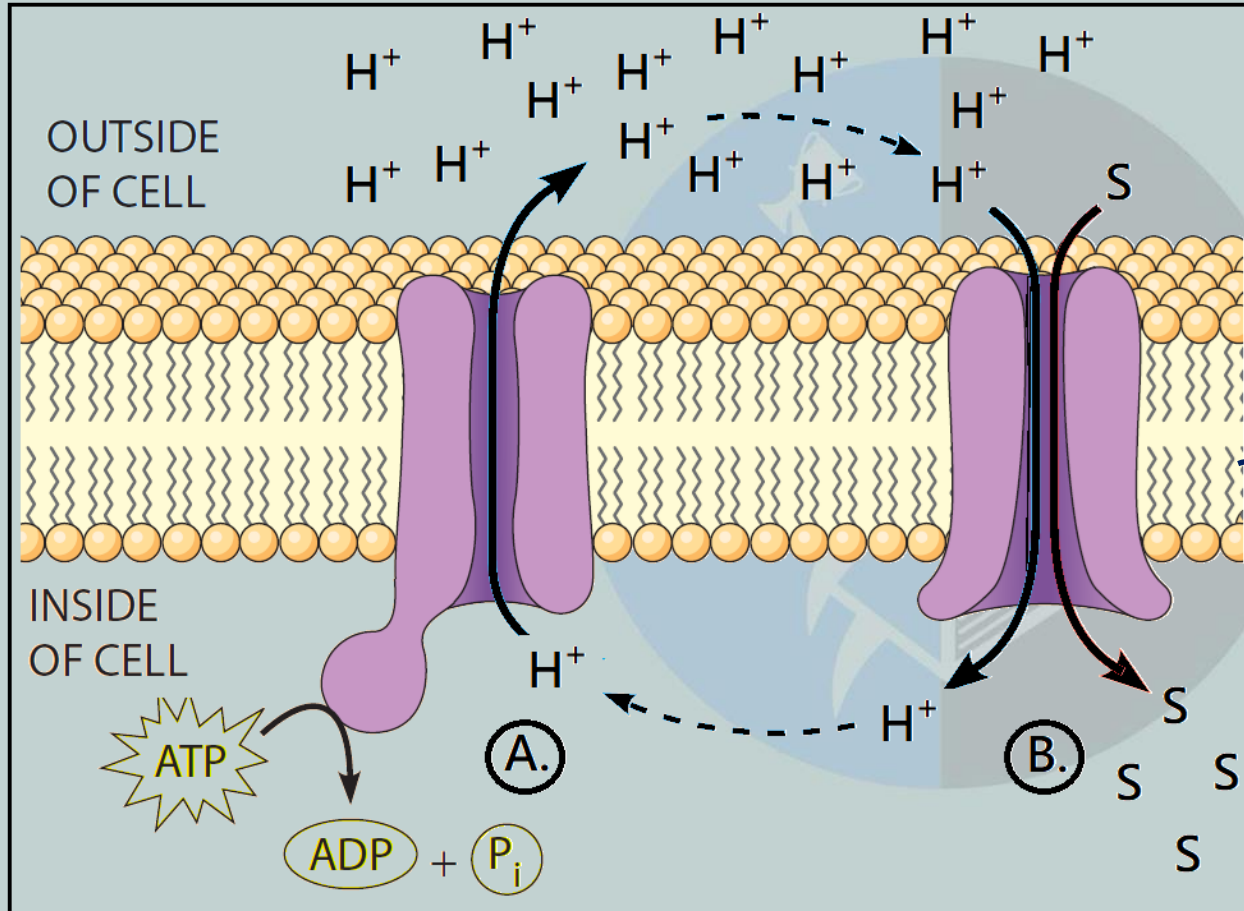
Lactose - H^+ - Permiase

Symporter

- Lactose - Against Gradient
- H^+ - Along Gradient

Bacterial P.m $\rightarrow$





Symporter

Bacterial P.m.



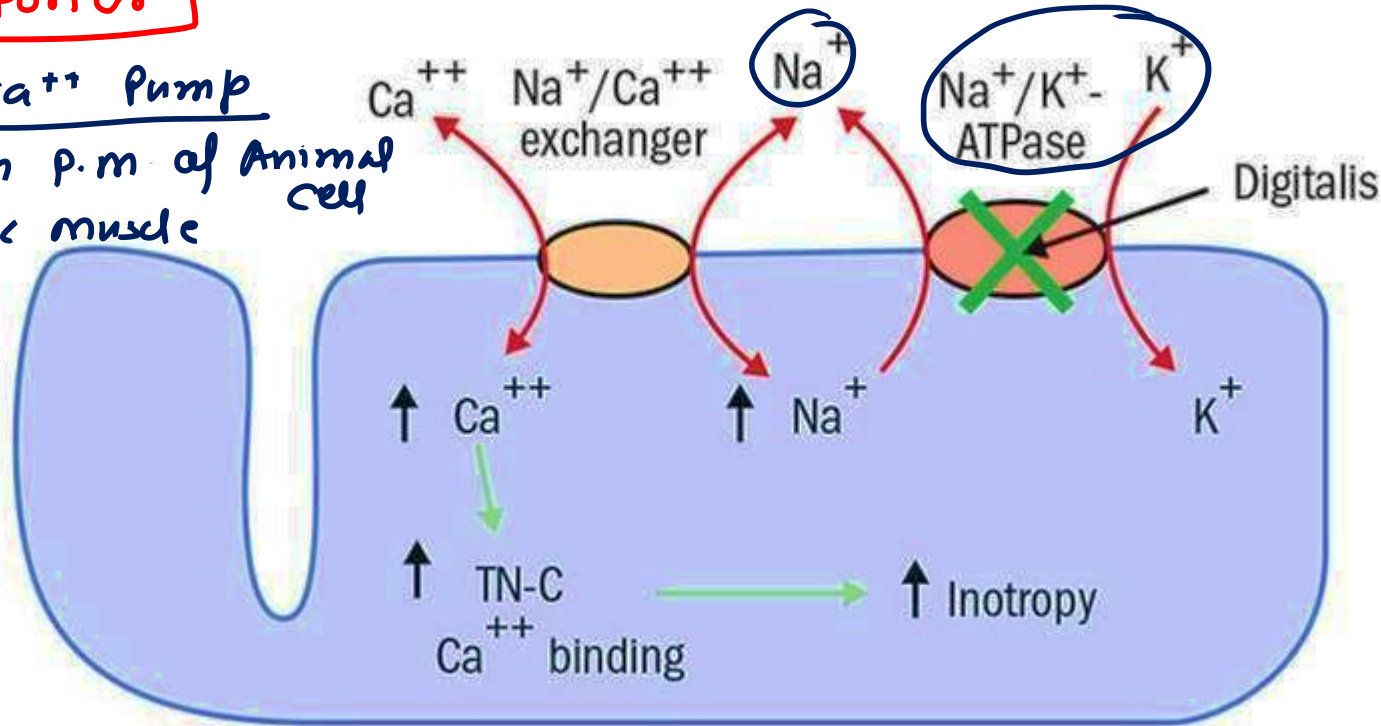
Symporter – secondary active transporter

- SGLT-1 – Na^+ - Glu symporter
- Lactose- permease (E. coli)
- H^+ Pyruvate transporter (present on inner membrane of mitochondria)
- H^+ - iP transporter (present on inner membrane of mitochondria)
- H^+ - peptide transporter (intestinal epithelial Apical side –(Pep t -1)
- Na^+ - Iodine Transporter – Epithelial cells of thyroid gland

Antiporter -

$\text{Na}^+ - \text{Ca}^{++}$ Pump

- +nt on p.m of Animal cell
- Cardiac muscle





Primary Active Transport

This involves direct use of ATP hydrolysis.

Generates electrochemical gradient

Example: Na⁺-K⁺ Pump

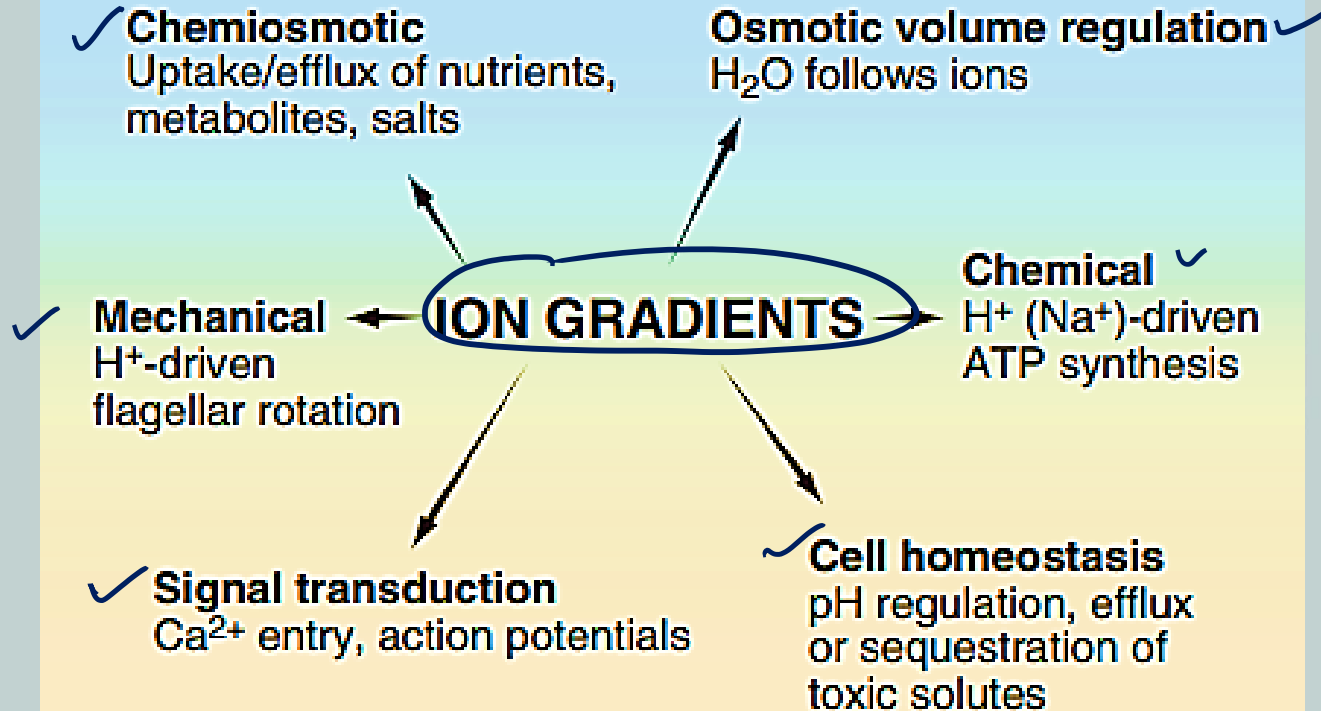
Secondary Active Transport

Utilizes electrochemical gradient generated by primary active transport as energy input.

Dissipates electrochemical gradient

Example: Na⁺ -Ca²⁺ Pump

$$\Delta V = 2.303 \frac{RT}{ZF} \log \frac{[C_{out}]}{[C_{in}]}$$





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L1: DNA Replication

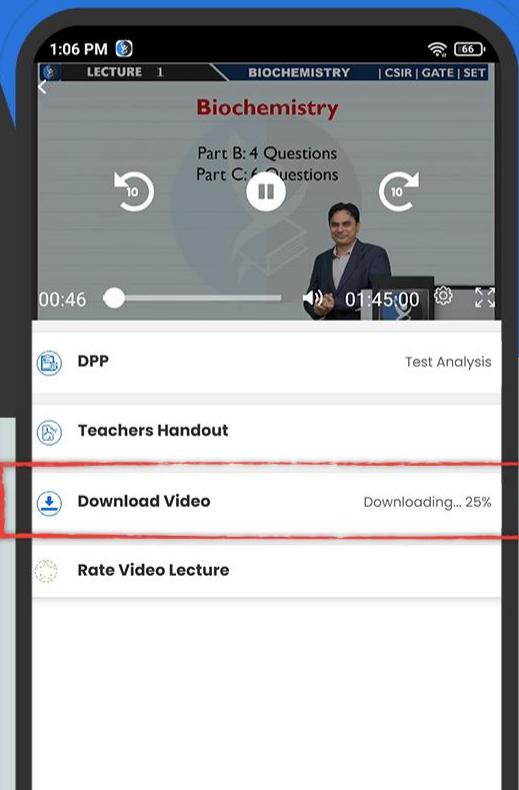


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